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Electrochemical ELISA using diamond-like carbon (DLC) microelectrode for RA diagnosis

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Abstract

A diamond-like carbon (DLC) microelectrode was applied for diagnosis of RA (rheumatoid arthritis). In this work, oxidized 3,5,3',5'-tetramethylbenzidine (TMB) was quantified by amperometry instead of optical density measurement. Quantification was carried out by using a boron doped DLC microelectrode and cyclic voltammetric analysis method without quenching step which uses sulfuric acid. For the RA diagnosis, the cut-off value was determined by considering the sensitivity as well as the selectivity of the analysis. The test results demonstrate that electrochemical ELISA using DLC microelectrode was feasible for the RA diagnosis

Keywords: Diamond-like carbon, elctrochemical ELISA, microelectrode, RA

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) has been applied to the diagnosis of various diseases. In commercial ELISA, a target analyte is usually quantified from a chromogenic reaction of TMB. From the previous study, we demonstrated that boron-doped DLC microelectrode can be applied to commercial ELISA kits for detection of HIV antigen, HBV antigen and HCV antigen is feasible1. The boron-doped diamond-like carbon is known to have an electrochemical window of -1.5V to +2.5V against Ag/AgCl reference electrode. Such a DLC electrode can e used for the amperometric analysis of TMB in commercial ELISA kits within the electrochemical window between -1.0V to +1.0V against Ag/AgCl reference electrode. In this work, feasibility of using boron-doped DLC microelectrodes for diagnosis of RA was demonstrated.

2. Materials and method

2.1. DLC microelectrode

The DLC microelectrode is composed on working electrode (WE), counter electrode (CE) and reference electrode (RE) as shown in Fig. 1. The DLC working electode was deposited as boron-doped DLC on a silicon

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wafer, then another carbon layer was deposited as a passivation layer. The coounter electrode was made of platinum and located around the working electrode. The reference electrode was a sintered Ag/AgCl powder.

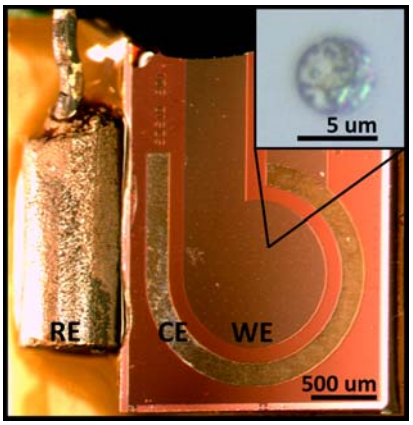

Fig. 1: Diamond-like carbon (DLC) microelectrode. Working, Ag/AgCl reference and counter electrodes are noted as WE, RE and CE, respectively. The enclosed picture shows the disc-type working electrode with a diameter of 5μ m.

2.2. Cyclic voltammetric measurment

The cyclic voltammetry of TMB was performed at the potential range of -0.2 to +0.7V against Ag/AgCl at the scanning rate of 0.1V/s. The cyclic voltammetric measurement was repeated three times and the 3rd data were took as signal analysis.

3. Results and discussion

3.1. Quantitative analysis of oxdized TMB

Many commercial ELISA kits have been used TMB as a chromogenic substrate of horseradish peroxidase (HRP)[2]. The TMB has well known two-step oxidation profile and the color developing reaction of TMB is carried out as follows: TMB (colorless) → ox-TMB1(blue) → ox-TMB2(yellow). In order to demonstrate that DLC microelectrode can be applied to ELISA based on the TMB reaction, the electrode should be able to detect the oxidized TMB molecules. As shown in Fig. 2, step-wise conversion of TMB can be observed bt cyclic voltammetric analysis. The microelectrode gives a well-known, narrow and step-shaped graph as shown in Fig. 2, and limit of detection (LOD) could be improved by clear determination of reductive and oxidatuve peaks[3].

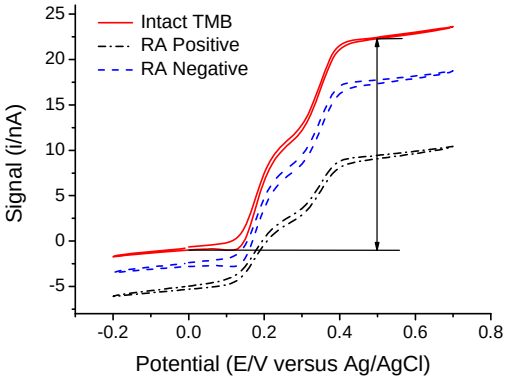

Fig. 2: Cyclic voltammogram (CV) of TMB analysis after ELISA test with positive and negative RA patient sera.

3.2. Correlation curve for the quatification of TMB

In this work, the conecntration of oxidized TMB was measured by using the DLC microelectrode without the acid quenching step. The signal from the DLC microelectrode of ox-TMB1 was plotted with respect to the optical density value at 650nm with a reference wavelength of 450nm as shown in Fig. 3. The correlation curve shows linearity of 0.989 and the average deviation was 5.33%.

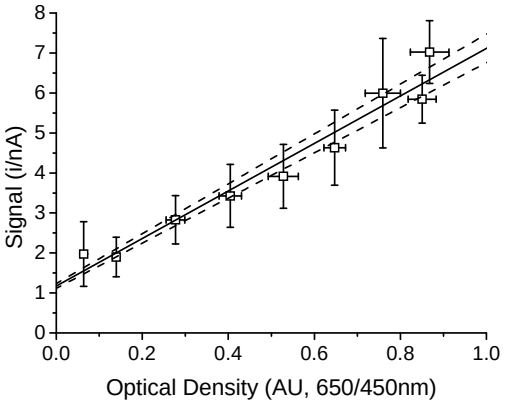

Fig. 3: The correlation curve between the electrical signals and the optical densities (OD). Dash lines represent the standard deviations.

3.3. Application to RA diagnosis

The DLC microelectrode was applied to RA diagnosis based on ELISA test. For the test, RA positive sera (n=23) and normal sera (n=7) were tested. In this work, measuring of OD and CV signals were done before the acid quenching step. The amount of ox-TMB1 was calculated from the CV as shown in Fig.2.

In this work, the cut-off value was set by ROD curve (data is not shown) and the value was 7.3 There were 7 positive patients in a RA positive group and none of normals above the cut-off as shown in Fig. 4 (a) and (b). From the results, the sensitivity and the selectivity were calculated as 44% and 100%, repectively.

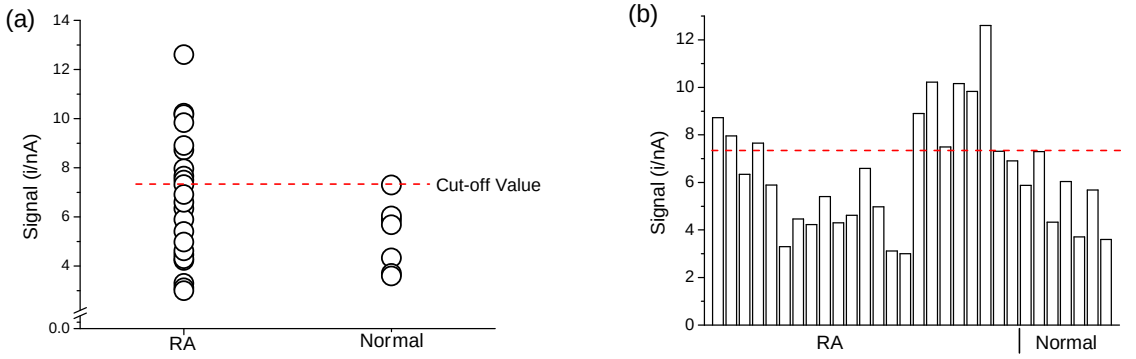

Fig. 4: RA diagnosis by establishing an optimal cut-off value. (a) Determination of cut-off value. Horizontal line represents the cut-off value at the optimum sensitivity and selectivity. (b) Anti-CCP electrochemical ELISA test result with RA patient sera.

4. Conclusion

In this work, the electrochemical ELISA based on DLC microelectrode as shown in Fig. 1 was applied to RA diagnosis by the detection of anti-CCP (cyclic citrullinated peptide) antibodies which were known to be found in the serum of RA patients[4]. For the detection of anti-CCP antibodies, conventional ELISA was performed by using TMB as a chromogenic substrate. The current difference between at 0V and 0.5V was used as signals as seen in Fig. 2, and the signal difference between intact TMB and samples were used as data. The relationship between optical density and the electrical signal is shown in Fig. 3, and the electrical signals were found to have linear correlation to the optical densities (OD) at the wavelength of 450 nm. By using this correlation curve for the amperometric signals from analysis of TMB, DLC electrode can be applied for the conventional ELISA based on TMB without any modification.

For the RA diagnosis, the cut-off value was determined as shown in Fig. 4 by considering the sensitivity as well as the selectivity of the analysis. The test results demonstrate that electrochemical ELISA using DLC microelectrode was feasible for the RA diagnosis.

Acknowledgements

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